CONTROL OF AMINOENDOPEPTIDASE AND ALKALINE PHOSPHATASE SYNTHESIS IN *ESCHERICHIA COLI*: EVIDENCE FOR A REGULATION BY INORGANIC PHOSPHATE-INDEPENDENT OF *pho R* AND *pho B* PHOSPHATASE REGULATORY GENES

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1. Introduction

We have previously presented evidences for the presence of an aminoendopeptidase located near the cell surface of *Escherichia coli* [1] and other Gram-negative organisms (unpublished results). Although this enzyme is constitutively produced, the differential rate of synthesis is increased about 4-fold simultaneously with derepression of alkaline phosphatase synthesis upon phosphate starvation [1,2]. This effect is specific for inorganic phosphate (P_i); starvation for nitrogen or for the carbon and energy source does not cause any increase [1]. The effects of the mutations involved in the control of alkaline phosphatase synthesis have been investigated [2]. *pho R* gene product does not seem to be required either for the constitutive or for the derepressed synthesis of aminoendopeptidase. Mutations in *pho S* and *pho T* influence the intracellular level of P_i in much the same way as depletion of P_i from the growth medium, and only as a consequence influence the synthesis of aminoendopeptidase and alkaline phosphatase. In this paper, we have analyzed the effect of the *pho B* gene product [3]. This latter has been proposed to code for an activator protein which is necessary for the expression of the *pho A* gene (structural gene of alkaline phosphatase), and three other genes coding for 3 periplasmics proteins that are also repressed by inorganic phosphate [4,5]. A genetic analysis has recently shown a positive control [6].

We did not find any effect of the *pho B* gene product on the aminoendopeptidase synthesis. This, as well as other evidences previously reported [2], suggest that aminoendopeptidase is not one of the three periplasmic proteins that are derepressed in addition to alkaline phosphatase upon phosphate starvation. By using a thermosensitive mutant previously isolated by Gallant and Stapleton [7] we have obtained evidences that the same gene product regulates the 'derepression' of both enzymes at low P_i concentrations independently of *pho B* and *pho R*.

2. Materials and methods

2.1. *Bacterial strains and growth conditions*

The strain LEP_1, F^- *pho B lac pro C tsx pur E str trp thi has* been obtained from Dr Yagil. The strain PR_1A_2 is a 5-bromouracil induced mutant of *E. coli* B_3 which has been obtained from Dr Gallant. Cultures were grown at the temperature required in a tris-bactopeptone medium with 0.4% glucose as a source of carbon and energy. In this medium the inorganic phosphate is provided by the bactopeptone added and is limiting for growth as previously shown [1].

2.2. *Chemicals*

p-Nitrophenyl-phosphate was obtained from Sigma Chemical Co; L-alanine-*p*-nitroanilide was purchased from Bachem Inc. All other reagents used were of the best available grade.

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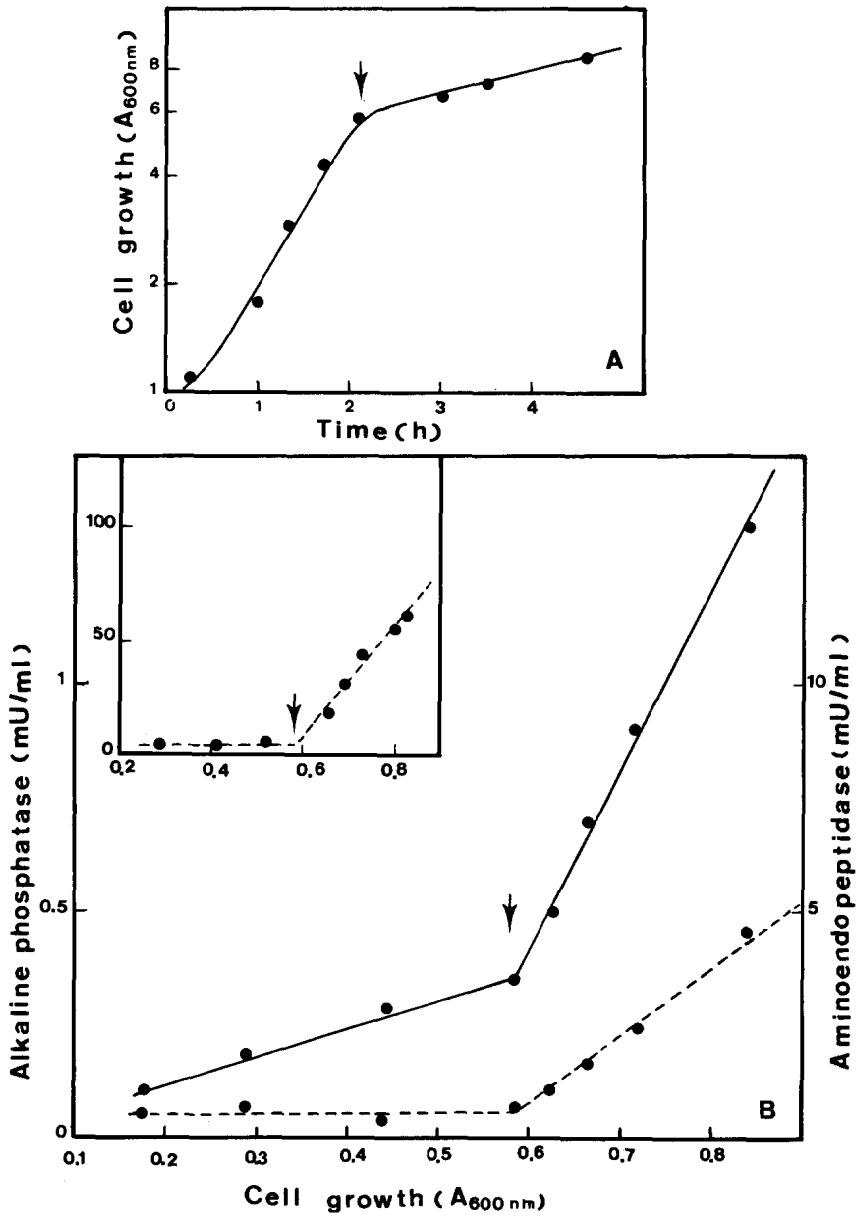

Fig.1. Effect of mutation in *pho B* on aminoendopeptidase synthesis. (A) Growth curve of the culture; at the cell density indicated by the arrow the growth became limited for P_i. (B) The differential rates of base alkaline phosphatase synthesis (– –) and of aminoendopeptidase synthesis (—) were followed. (Inset) For comparison, we have shown a plot of the derepression of alkaline phosphatase in a wild-type strain grown under identical conditions as LEP_1.

2.3. *Assay for enzymic activity*

The procedures for measuring alkaline phosphatase and aminoendopeptidase activities have been described previously [1].

3. Results

3.1. *Effect of mutation in* pho B *on aminoendopeptidase synthesis*

LEP_1 is a phosphatase-negative mutant strain which does not map in the *pho A* gene. Bracha and Yagil [3] have shown that it is altered in a closely linked gene that they called *pho B*.

When this mutant was grown on the Tris-bacto-peptone medium where growth is limited for P_i as previously described [1], we found the typical 'derepression' of aminoendopeptidase upon phosphate starvation (fig.1). As indicated by Bracha and Yagil [3] very little alkaline phosphatase was detected. However, by assaying the activity during overnight incubation after checking the linearity of the hydrolysis of the substrate, we did found an increase of the base synthesis of alkaline phosphatase upon P_i starvation. (fig.1).

3.2. *Evidence for the involvement of the same gene product in 'derepression' of alkaline phosphatase in* pho R *or* pho B *mutants and of aminoendopeptidase in wild type strains*

PR_1A_2 is a mutant strain which is thermosensitive for derepression of alkaline phosphatase [7]. At 25°C the synthesis of this enzyme is normally repressed by P_i, whereas shifting the cultures to 39°C causes a derepression (fig.2). Under these conditions, the culture at both temperatures grew exponentially and was not limited for P_i during the part of the growth that was followed. One observed a simultaneous derepression of alkaline phosphatase and of aminoendopeptidase when the culture was shifted from 25°C to 39°C.

4. Discussion

In previous studies our attention was called by the fact that a simultaneous 'derepression' of alkaline phosphatase and of aminoendopeptidase occured upon P_i limitation of growth not only in wild-type strains but also in *pho R* mutants. In this work we show that *pho B* mutants behave in the same way. This suggests that the regulation by inorganic phosphate we are interested in, is independent of *pho R* and *pho B* gene product. Thus, a novel regulatory mechanism under P_i control yet undescribed seems to be operating. The occurence of this regulatory element might be of a rather general interest since we found it in all gram-negative organisms tested (submitted for publication).

In the course of a genetic study recently published [6] of the *pho B* gene product regulation, Brickman and Beckwith have noticed that in *pho* A^+ *pho* B^- strains, a 4-fold derepression of phosphatase levels occured under conditions of phosphate limitation. This result is in agreement with ours and does reflect a separate control over the basal level of alkaline phosphatase.

In derivatives of *E. coli* K12, the basal level of this enzyme has been shown to be 10-fold higher than in derivatives of strain *E. coli* B [8]. In the course of this investigation, mapping experiments indicated that genes responsible for the regulation of the basal level lie within *xyl-rha* region of the chromosome adjacent to the *pho S* locus. The results obtained with the mutant PR_1A_2 suggest that the same gene product is involved in the regulation of phosphatase and aminoendopeptidase synthesis observed. The mutant PR_1A_2 has been reported to carry a mutation in *pho S*, however no precise mapping has been published [9]. Furthermore, *pho S* mutants have been shown to be affected in phosphate transport [10] and such a defect has not been observed either by Gallant et al. [7] or in our laboratory. This might be due to the fact that the PR_1 mutation is close to *pho S* in the *xyl–rha* region that regulates the basal level of alkaline phosphatase. Then, one could easily understand the co-regulation of phosphatase and aminoendopeptidase synthesis at 40°C in the mutant PR_1A_2. The structure or the biosynthesis [9] of the gene product that normally interact with P_i (or a related compound) and mediates the regulation of basal phosphatase and aminoendopeptidase synthesis would be altered at 40°C. This could prevent the interaction and cause the temperature sensitive 'derepression' observed.

We are currently testing this hypothesis as well as trying to isolate other regulatory mutants to improve our understanding of this regulation.

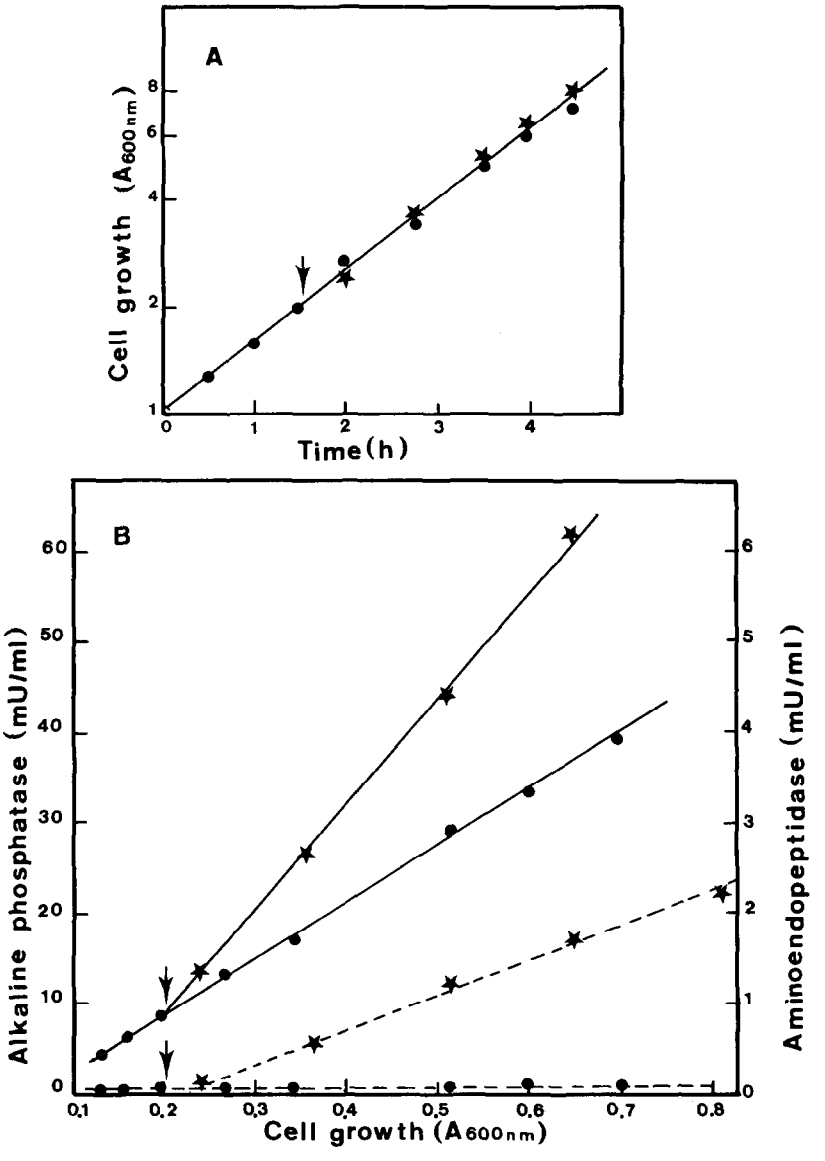

Fig.2. Evidence for a co-regulation of base synthesis of alkaline phosphatase and aminoendopeptidase synthesis. (A) Growth curve of the culture. At the time indicated by the arrow the culture was divided, one part was shifted to 39°C (★–★) the other one was kept at 25°C (●–●) as a control. (B) The differential rates of synthesis of alkaline phosphatase (– – –) and aminoendopeptidase (——) were followed at 25°C (●–●) and 39°C (★–★).

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